REMARKS

The Official Action of December 13, 2007 and the references cited therein have been carefully considered. The Applicant respectfully requests reconsideration of the application in view of the following remarks. The Specification has been amended to ensure that the provisional application US Application No. 60/419,203, filed October 17, 2002, is appropriately mentioned in this application which entered the US National Phase under 35 U.S.C. § 371. In this regard, Applicants note that the Filing Receipt for the subject application already correctly references the Provisional application US Application No. 60/419,203, filed October 17, 2002.

Claims 1-50 have been canceled without prejudice and new Claims 51-60 have been added to be directed to a specific aspect of the present invention. Support for this amendment is found on page 4, lines 29-25; page 6, line 5 to page 8, line 10; and in the claims of the application as filed. Claims 51-60 are pending in the application.

I. Rejection of Claims 33-50 under 35 U.S.C. § 112, First Paragraph

Claims 33-50 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. Applicants respectfully traverse this rejection and submit that, in view of the specification and the state of the art, one of ordinary skill in the art could practice the claimed invention without undue experimentation.

With respect to the Nature of the Invention, the present claims are directed to a method for reducing the number of awakenings during sleep in a mammalian patient by using a T-type calcium channel antagonists that have a specified selectivity and potency, i.e. selectivity for the T-type calcium channel relative to the L-type calcium channel of at least 100 fold, selectivity for the $\alpha 1I$ subtype T-type calcium channel relative to the $\alpha 1G$ subtype T-type calcium channel of at least 10 fold, selectivity for the $\alpha 1I$ subtype T-type calcium channel relative to the $\alpha 1H$ subtype T-type calcium channel of at least 10 fold, and potency of an IC_{50} for binding to the T-type calcium channel of 500 nM or less. Accordingly, the nature of the invention is that it is directed to the use of compounds having specific, ascertainable properties for effecting a specific, ascertainable therapeutic outcome.

With respect to the Breadth of the Claims, Applicants note that the present invention is not directed to particular compounds per se. The present claims are directed to the use of compounds having specific, ascertainable properties for effecting a specific, ascertainable therapeutic outcome.

With respect to Guidance of the Specification and Working Examples, Applicants note that the claims are directed to the use of T-type calcium channel antagonists having a specified selectivity for reducing the number of awakenings during sleep in a mammalian patient. In this regard, the preclinical data in Example 2 demonstrates that T-type calcium channel antagonists reduce the number of awakenings during sleep relative to vehicle.

The Examiner indicated that no structures were provided for the compounds A-C in the table on page 17. Applicants note that although these compounds vary widely in their chemical structures, they have a common ability to selectively inhibit T-type calcium channel function and be useful in enhancing sleep. Applicants note that these compounds are more fully described on page 19 of their provisional application USSN 60/419,203, filed October 17, 2002:

Compound B: 2-(R)-(1-(R)-(3,5-bis(trifluoromethyl)phenyl)ethoxy)4-(5-(dimethyl-amino)methyl-1,2,3-triazol-4-yl)methyl-3-(S)-(4-fluorophenyl)morpholine (US 5,612,337)

T-type IC50	L-type IC50
~50 nM	> ~700nM

Compound C: 2-methoxy-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl]-([2S,3S]-2-phenyl-piperidin-3-yl)-amine (US 5,703,240, U.S. 5,843,966)

T-type IC50	L-type IC50
~200 nM	> ~2 uM

Compound D: (1S,2S)-2-(2-((3-(2-benzimidazolylpropyl)methylamino)ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphthyl methoxyacetate (US 4,808,605)

T-type IC50	L-type IC50
~2.7 uM	~19 uM

With respect to the State of the Art, Applicants direct the Examiner's attention to the enclosed reference (Lory, et al., Expert Opin. Ther. Targets (2007), 11(5) 717-722). As noted on page 718 therein, although mibefradil was initially believed to be a selective T-type calcium channel antagonist, it is now admitted that mibefradil potently inhibits many other ion channels, including L-type calcium channels as well as store-operated calcium channels.

With respect to the Nature and predictability of the invention, Applicants note that the specification demonstrates that selective T-type calcium channel antagonists possess physiological activity and are useful in accordance with the claimed invention for reducing the number of awakenings during sleep in a mammalian patient.

With respect to the Quantity of Experimentation necessary, Applicants note that methods to select the subject compound and formulate and administer it to reduce the number of awakenings during sleep in a mammalian patient are fully described in the specification.

Accordingly, the rejection of Claims 33-50 under 35 U.S.C. §112, first paragraph, for lack of enablement is untenable and should be withdrawn.

II. <u>Rejection of Claims 33-36 under 35 U.S.C. § 103(a)</u>

Claims 33-36 stand rejected under 35 U.S.C. § 103(a) as being obvious over Snutch et al.

The Applicants respectfully traverse this rejection and provide the following comments. The Applicants respectfully assert that Snutch et al. does not disclose or suggest the claimed invention. Nor would Snutch et al. have motivated or enabled one skilled in the art to employ the subject compounds in accordance with the claimed invention. Moreover, in view of the state of the art, one skilled in the art would have been discouraged from the compounds of the claimed invention.

Snutch et al. disclose sequences that encode T-type calcium channel subunits α1G subtype, α1H subtype and α1I subtype. Snutch et al. disclose that these subunits can be used to prepare functional calcium channels incorporating these subunits which can be used to evaluate the effects of pharmaceuticals for treating conditions where undesirable T-type calcium channel activity is present, including epilepsy, sleep disorders, mood disorders, cardiac hypertrophy and arrythmia and hypertension.

As the Examiner noted, Snutch et al. do not disclose the specific nature of the sleep disorder (e.g. insomnia, narcolepsy, etc.). Snutch et al. do not indicate whether a T-type calcium channel antagonist would be useful in a sleep disorder where enhancement of sleep is desired (e.g. insomnia) or a sleep disorder where wakefulness is desired (e.g. narcolepsy). In addition, Snutch et al. do not indicate which of the T-type calcium channel subunits α1G subtype, α1H subtype and α1I subtype would be an appropriate target for enhancement of sleep, let alone treating a sleep disorder, in general.

In contrast, the present claims are directed to a method for reducing the number of awakenings during sleep in a mammalian patient by using a T-type calcium channel antagonists that have a specified selectivity and potency, i.e. selectivity for the T-type calcium channel relative to the L-type calcium channel of at least 100 fold, selectivity for the α1I subtype T-type calcium channel relative to the α1G subtype T-type calcium channel of at least 10 fold, selectivity for the α1I subtype T-type calcium channel relative to the α1H subtype T-type calcium channel of at least 10 fold, and potency of an IC_{50} for binding to the T-type calcium channel of 500 nM or less.

Applicants respectively submit that there would have been no motivation nor guidance in Snutch et al. for one of ordinary skill in the art to have attempted to reduce the number of awakenings during sleep in a mammalian patient by using a T-type calcium channel antagonists that have a specified selectivity and potency in accordance with the claimed invention.

In fact, Snutch et al. teach away from the present invention by suggesting that activity at all of the subunits α1G subtype, α1H subtype and α1I subtype would have been desired.

Accordingly, Applicants respectfully submit that the rejection of Claims 33-36 under 35 U.S.C. § 103(a) as being obvious over Snutch et al. is untenable and should be withdrawn.

III. Rejection of Claims 37 and 49-50 under 35 U.S.C. § 103(a)

Claims 37 and 49-50 stand rejected under 35 U.S.C. § 103(a) as being obvious over Snutch et al. in view of Massie et al.

The Applicants respectfully traverse this rejection and provide the following comments. The Applicants respectfully assert that Snutch et al. in view of Massie et al. does not disclose or suggest the claimed invention. Nor would Snutch et al. in view of Massie et al. have motivated or enabled one skilled in the art to employ the subject compounds in accordance with the claimed invention. Moreover, in view of the state of the art, one skilled in the art would have been discouraged from the compounds of the claimed invention.

Snutch et al. disclose sequences that encode T-type calcium channel subunits α1G subtype, α1H subtype and α1I subtype. Snutch et al. disclose that these subunits can be used to prepare functional calcium channels incorporating these subunits which can be used to evaluate the effects of pharmaceuticals for treating conditions where undesirable T-type calcium channel activity is present, including epilepsy, sleep disorders, mood disorders, cardiac hypertrophy and arrythmia and hypertension.

Massie allegedly discloses that mibefradil selectively blocks T-type calcium channels. In this regard, Applicants direct the Examiner's attention to the enclosed reference (Lory, et al., <u>Expert Opin. Ther. Targets</u> (2007), 11(5) 717-722). As noted on page 718 therein, although mibefradil was initially believed to be a selective T-type calcium channel antagonist, it is now admitted that mibefradil potently inhibits many other ion channels, including L-type calcium channels as well as store-operated calcium channels.

As the Examiner noted, Snutch et al. do not disclose the specific nature of the sleep disorder (e.g. insomnia, narcolepsy, etc.). Snutch et al. do not indicate whether a T-type calcium channel antagonist would be useful in a sleep disorder where enhancement of sleep is desired (e.g. insomnia) or a sleep disorder where wakefulness is desired (e.g. narcolepsy). In addition, Snutch et al. do not indicate which of the T-type calcium channel subunits α1G subtype, α1H subtype and α1I subtype would be an appropriate target for enhancement of sleep, let alone treating a sleep disorder, in general.

In contrast, the present claims are directed to a method for reducing the number of awakenings during sleep in a mammalian patient by using a T-type calcium channel antagonists that have a specified selectivity and potency, i.e. selectivity for the T-type calcium channel relative to the L-type calcium channel of at least 100 fold, selectivity for the α1I subtype T-type calcium channel relative to the α1G subtype T-type calcium channel of at least 10 fold, selectivity for the α1I subtype T-type calcium channel relative to the α1H subtype T-type calcium channel of at least 10 fold, and potency of an IC_{50} for binding to the T-type calcium channel of 500 nM or less.

Applicants respectively submit that there would have been no motivation nor guidance in Snutch et al. in view of Massie et al. for one of ordinary skill in the art to have attempted to reduce the number of awakenings during sleep in a mammalian patient by using a T-type calcium channel antagonists that have a specified selectivity and potency in accordance with the claimed invention.

In fact, Snutch et al. in view of Massie et al. would have taught away from the present invention because Snutch et al. would have suggested that activity at all of the subunits α1G subtype, α1H subtype and α1I subtype would have been desired and Massie et al. would have suggested that activity at both the T-type calcium channel and the L-type calcium channel would have been desired.

Accordingly, Applicants respectfully submit that the rejection of Claims 37 and 49-50 under 35 U.S.C. § 103(a) as being obvious over Snutch et al. in view of Massie et al. is untenable and should be withdrawn.

IV. Rejection of Claims 41 and 43-47 under 35 U.S.C. § 103(a)

Claims 41 and 43-47 stand rejected under 35 U.S.C. § 103(a) as being obvious over Snutch et al. in view of Santi et al.

The Applicants respectfully traverse this rejection and provide the following comments. The Applicants respectfully assert that Snutch et al. in view of Santi et al. does not disclose or suggest the claimed invention. Nor would Snutch et al. in view of Santi et al. have motivated or enabled one skilled in the art to employ the subject compounds in accordance with the claimed invention. Moreover, in view of the state of the art, one skilled in the art would have been discouraged from the compounds of the claimed invention.

Snutch et al. disclose sequences that encode T-type calcium channel subunits α1G subtype, α1H subtype and α1I subtype. Snutch et al. disclose that these subunits can be used to prepare functional calcium channels incorporating these subunits which can be used to evaluate the effects of pharmaceuticals for treating conditions where undesirable T-type calcium channel activity is present, including epilepsy, sleep disorders, mood disorders, cardiac hypertrophy and arrythmia and hypertension.

Santi et al. disclose that certain neuroleptic agents have activity at T-type calcium channels. Most of these agents are not selective for a particular subtype of the subunits α1G subtype, α1H subtype and α1I subtype. Flunarizine is disclosed as having a preferential block of the α1G subtype and α1I subtype compared with the α1H subtype.

As the Examiner noted, Snutch et al. do not disclose the specific nature of the sleep disorder (e.g. insomnia, narcolepsy, etc.). Snutch et al. do not indicate whether a T-type calcium channel antagonist would be useful in a sleep disorder where enhancement of sleep is desired (e.g. insomnia) or a sleep disorder where wakefulness is desired (e.g. narcolepsy). In addition, Snutch et al. do not indicate which of the T-type calcium channel subunits α1G subtype, α1H subtype and α1I subtype would be an appropriate target for enhancement of sleep, let alone treating a sleep disorder, in general.

Santi et al. do not disclose that their particular neuroleptic agents are useful for educing the number of awakenings during sleep in a mammalian patient. Most of these neuroleptic agents are not selective for a particular subtype of the subunits α1G subtype, α1H subtype and α1I subtype. Only flunarizine is disclosed as having a preferential block of the α1G subtype and α1I subtype compared with the α1H subtype.

In contrast, the present claims are directed to a method for reducing the number of awakenings during sleep in a mammalian patient by using a T-type calcium channel antagonists that have a specified selectivity and potency, i.e. selectivity for the T-type calcium channel relative to the L-type calcium channel of at least 100 fold, selectivity for the α1I subtype T-type calcium channel relative to the α1G subtype T-type calcium channel of at least 10 fold, selectivity for the α1I subtype T-type calcium channel relative to the α1H subtype T-type calcium channel of at least 10 fold, and potency of an IC_{50} for binding to the T-type calcium channel of 500 nM or less.

Applicants respectively submit that there would have been no motivation nor guidance in Snutch et al. in view of Santi et al. for one of ordinary skill in the art to have attempted to reduce the number of awakenings during sleep in a mammalian patient by using a T-type calcium channel antagonists that have a specified selectivity and potency in accordance with the claimed invention.

In fact, Snutch et al. in view of Santi et al. would have taught away from the present invention because Snutch et al. would have suggested that activity at all of the subunits α1G subtype, α1H subtype and α1I subtype would have been desired and Santi et al. would have reinforced Snutch et al. by suggesting that activity at all of the subunits α1G subtype, α1H subtype and α1I subtype would have been desired, or in the case of flunarizine that preferential block of the α1G subtype and α1I subtype compared with the α1H subtype would have been desired.

Accordingly, Applicants respectfully submit that the rejection of Claims 41 and 43-47 under 35 U.S.C. § 103(a) as being obvious over Snutch et al. in view of Santi et al. is untenable and should be withdrawn.

Applicants respectfully contend that the application is allowable and a favorable response from the Examiner is earnestly solicited.

Respectfully submitted,

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Expert Opinion

General

Towards the discovery of novel T-type calcium channel blockers

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Despite their presence in many tissues and their potential implication in various disease states, low-voltage activated T-type calcium channels (T-channels) have only recently become targets of interest. Unfortunately, the lack of selective T-channel blockers has hampered further characterisation of these channels. The recent availability of cloned T-channels, the Ca_V3 proteins, facilitates identification of novel T-channel blockers. Also, studies performed in knockout animals have fostered novel interest. Selective inhibition of T-channels may have clinical importance in cardiovascular diseases, some forms of epilepsy, sleep disorders, pain and possibly cancer. This review focuses on novel research approaches to discover potent and selective T-channel modulators. These molecules may be potential drugs for treating human diseases, as well as important tools to decipher the physiological role of these channels.

Keywords: Ca_V3 subunit, low-voltage activated, T-type calcium channel

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1. T-type calcium channels in brief

T-type calcium channels (T-channels) belong to the family of voltage-gated calcium channels (VGCCs). The electrophysiological hallmarks of the T-channels are well established: low-voltage activated calcium current, fast (transient) inactivation kinetics and low unitary conductance. The properties of these channels are presented in great detail in several reviews [1-4]. Three genes, *CACNA1G*, *CACNA1H* and *CACNA1I*, code for the pore-forming subunits of T-channels, named $Ca_V3.1$ (α_{1G}), $Ca_V3.2$ (α_{1H}) and $Ca_V3.3$ (α_{1I}) subunits, respectively. Calcium entry through T-channels mediates membrane depolarisation and increase in intracellular calcium concentration that are thought to contribute significantly to pacemaker activities in the heart and in neurons, sleep, hormone secretion, mechanosensation, epilepsy and pain (for a recent overview see [5]). Until recently, the physiological role of T-channels has remained elusive. Mostly, hypotheses regarding putative roles of these channels were formulated according to the presence of T-type currents (T-currents), but no selective blocker of T-channels exists to unequivocally demonstrate the role(s) of any of the Ca_V3 subunits. Therefore, knockout animals [6,7] have provided important clues into the role of T-channels in neuronal and cardiovascular functions. As an example, inactivation of *cacna1g* in mice results in resistance to absence seizures [6], hyperalgesia to chronic pain [8], sleep instability [9] and slowing of the heartbeat [10].

2. History of T-channel pharmacology

Pharmacological studies of T-channels have balanced between hope and frustration. The original description of mibefradil being a T-channel blocker [11] has

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significantly fostered interest in the field (reviewed in [12]). Although more than half of the pharmacological studies on T-channels rely on mibefradil, investigators are still awaiting a selective T-channel blocker [5]. There is a considerable variability in T-channel pharmacology in native tissues, which has been attributed to the existence of T-channel isoforms. Cloning of the Ca_V3 subunits has enabled pharmacological analysis of T-channel isoforms. Indeed, the $Ca_V3.2$ channel is more sensitive than the $Ca_V3.1$ channel to several classical T-channel blockers, such as nickel and amiloride in heterologous expression systems, even though their electrophysiological properties are rather similar [2]. The pharmacology of $Ca_V3.3$ channel, which exhibits distinct electrophysiological properties and expression profile from $Ca_V3.1$ and $Ca_V3.2$ channels, has been less investigated.

Members of many classes of organic molecules: dihydropyridines, succinimide derivatives, diphenylbutylpiperidine derivatives, benzodiazepines, anesthetics and so on, which are currently used to treat a variety of neuronal and cardiovascular diseases, are inhibitors of the T-channels [2,3,13]. Several inorganic divalent and trivalent cations, as well as some toxins, are also T-channel blockers. Some of these T-channel blockers allow discrimination between T-type and other VGCC-related currents, but none are selective enough for T-channels. Although requiring caution for a correct interpretation of *in vivo* or *in situ* pharmacological data, the use of these drugs has offered insights into the physiological significance of T-channels in both normal and diseased cells. The authors anticipate that a new generation of more selective T-channel blockers will be crucial to foster their study and the treatment of a number of diseases that involve these channels.

3. Lessons from the 'classical' of T-channel blockers

3.1 Nickel (Ni^{2+})

Inorganic divalent cations, as well as trivalents cations [14], were of the first chemicals used to block T-currents. Most of these cations (i.e., Ni^{2+}) have limited use as T-channel antagonists, as they also attenuate calcium currents conducted by the various VGCC subtypes, as well as other ionic channels, although it is generally found that T-currents are more sensitive to Ni^{2+} than currents conducted by high-voltage activators [2]. Variability in the Ni^{2+} sensitivity of endogenous T-channels is well documented, and studies of recombinant T-channels demonstrate that Ni^{2+} is significantly more potent (> 10-fold) at inhibiting T-currents conducted by $Ca_V3.2$ than currents conducted by $Ca_V3.1$ or $Ca_V3.3$ in expression systems [15].

3.2 Mibefradil

In the early 1990s, mibefradil (Ro405967), which is a tetralol derivative structurally related to verapamil, showed promising antihypertensive and antianginal properties [16]. Mibefradil was marketed in 1997 as the first selective T-channel blocker, which stimulated considerable interest in the physiological and pathophysiological roles of these channels. After mibefradil was approved for clinical use, it was withdrawn from the market in 1998 due to drug interactions leading to irregular heart rhythms. It was shown that mibefradil inhibits CYP450 3A4 and 2D6, enzymes used to metabolise a number of therapeutic agents [17]. Although mibefradil remains a suitable pharmacological tool for T-channels, it is now admitted that mibefradil potently inhibits many other ion channels, including L-type calcium channels [18,19] as well as store-operated calcium channels [20].

3.3 Amiloride

The diuretic agent amiloride, which is a prototypic inhibitor of epithelial sodium channels, was depicted as a T-channel blocker in early studies [21,22]. Recent studies showed that amiloride preferentially inhibits T-current related to recombinant $Ca_V3.2$ channels [23] and has a moderate affinity for $Ca_V3.1$ channel [24], reconciling the data obtained in a variety of native cells [25-27].

3.4 Flunarizine, pimozide and other antipsychotic and neuroleptic drugs

Flunarizine, pimozide penfluridol and fluspirilene are antipsychotic drugs used clinically to treat a variety of psychiatric disorders. Flunarizine is a diphenyldiperazine derivative used as neuroleptic that has been described as one of the most potent organic blocker of neuronal T-channels [28]. Santi *et al.* [29] reported that unlike other neuroleptics, flunarizine preferentially blocks $Ca_V3.1$ and $Ca_V3.3$ channels (K_d = 0.53 and 0.84 µM, respectively), compared with $Ca_V3.2$ channels (K_d = 3.6 µM). Pimozide is an antipsychotic drug of the diphenylbutylpiperidine class. As with most antipsychotics and neuroleptics, pimozide is known to act by blocking dopaminergic D_2 receptors. Notably, pimozide blocks the recombinant Ca_V3 channels (~ 40 nM [29]) in the same concentration range as pimozide binds to D_2 receptors (K_d ~ 29 nM [30]), suggesting a block of T-channels in clinical situations. Ca_V3 channels are also blocked by penfluridol (IC_{50} ~ 110 nM) and haloperidol (~ 1 µM). Altogether, an overview of the published data indicate that neuroleptics may affect a variety of cellular targets, including T-channels, to alleviate symptoms of many psychiatric diseases.

3.5 Succinimides, phenytoin and other antiepileptics

Ethosuximide, trimethadione and mesuximide are used in clinics in the treatment of generalised (petit mal) absence seizures. Whether or not T-channels are the site of action of succinimides is still controversial [31,32]. The data reported suggest that succinimides act at multiple cellular sites of action to prevent spike and wave discharges. Phenytoin, which is used clinically to treat partial and generalised seizures, is known to primarily inhibit sodium channels. Recombinant T-channels are blocked at concentrations close to the maximal therapeutic concentration of phenytoin,

suggesting that inhibition of T-channels may contribute to its therapeutic action, especially considering that phenytoin preferentially acts on inactivated channels. Other antiepileptics, such as lamotrigine (phenyltriazine derivative) and sipatrigine, known for their neuroprotective and anticonvulsant properties, also inhibit T-channels, but at concentrations that may be clinically irrelevant [33].

3.6 Anesthetics

Although the precise mechanism of action of general anesthetics is unknown, many studies have demonstrated that they are capable of modulating the activity of T-channels. Many volatile anesthetics, such as enflurane, halothane and isoflurane, inhibit endogenous and recombinant T-channels at therapeutically relevant concentrations [25]. This block of T-channels may contribute to their anesthetic and analgesic properties, as in the case of nitrous oxide that potently inhibits $Ca_V3.2$ channels [34]. Barbiturates, such as pentobarbital and phenobarbital, are able to fully block recombinant and endogenous T-channels [35]. However, T-channels may not represent a therapeutic site of action for barbiturates as the concentrations needed to inhibit T-channels are significantly greater than those measured in clinical settings (reviewed in [25]).

3.7 Fluoxetine

Fluoxetine, a diphenhydramine derivative, is a psychoactive drug widely prescribed in depression. The therapeutic action of fluoxetine primary results from the inhibition of serotonin reuptake. Micromolar concentrations of fluoxetine affect T-channels [36], which are also markedly inhibited by the active metabolite norfluoxetine. Inhibition of T-channels represents a novel mechanism by which fluoxetine may be pharmacologically active, which could account for some of the clinical and/or side effects in treated patients. Fluoxetine inhibition of T-channels should, therefore, be taken into account in further studies investigating the pharmacological properties of this antidepressant.

4. Toxins active on T-channels

Kurtoxin is a 63 amino acid peptide isolated form the scorpion *Parabunthus transvaalicus* with high affinity for both $Ca_V3.1$ ($IC_{50} \sim 15$ nM) and $Ca_V3.2$ ($IC_{50} \sim 61$ nM) channels [37]. Unfortunately, this promising pharmacological tool for the study of T-channels was shown to also interact with L-, N- and P-channels in central and peripheral neurons [38].

Two related kurtoxins from the scorpion *Parabuthus granulatus*, named kurtoxin-like I and II, potently inhibit native T-channels, whereas KLI weakly blocked $Ca_V3.3$ channels expressed in *Xenopus* oocytes [39]. Overall, there is a great interest in finding natural ligands, especially toxins, which would selectively recognise and inhibit T-channels. Beyond purification, it is important to consider the possibility to produce recombinant toxins with increased selectivity using site-directed mutagenesis.

5. The search for endogenous blockers

5.1 Anandamide and other bioactive lipids

Anandamide (*N*-arachidonoyl ethanolamide) belongs to a major class of small lipid messengers, including endocannabinoids and *N*-acyl-related molecules, eicosanoids and fatty acids. These bioactive lipids are involved in neuronal excitability, sleep, epilepsy, neuroprotection, inflammation and pain, as well as cardiovascular modulation (reviewed in [40]). Of importance, anandamide directly blocks T-channels at submicromolar concentrations [41]. This inhibition represents a new non-cannabinoid receptor target likely to contribute to the wide variety of anandamide's effects. Notably, both the hydroxyl group and the level of unsaturation of the alkyl chain of anandamide critically impact T-channel inhibition [42]. Other polyunsaturated fatty acids and *N*-acyl ethanolamides that fullfill these criteria are, therefore, potent T-channel blockers [42-44]. These data indicate that T-channel inhibition may contribute to natural polyunsaturated fatty acid and *N*-acyl ethanolamide effects.

5.2 Zinc (Zn^{2+})

As reported above for Ni^{2+}, inorganic divalent cations have little interest as ion channel modulators, especially considering *in vivo* studies. However, some of these divalent cations, including Zn^{2+}, are pharmacological probes of physiological importance [45]. In the brain, Zn^{2+} is released from the presynaptic vesicles of glutamatergic neurons and free Zn^{2+} modulates many membrane receptors, transporters and channels, including T-channels that are inhibited by micromolar concentrations [46,47]. Zn^{2+} differentially modulates the three Ca_V3 channel isotypes [48] and preferentially inhibits $Ca_V3.2$ channels with an IC_{50} in the submicromolar range (~ 0.8 µM), which is 100 and 200-fold lower than that for $Ca_V3.1$ and $Ca_V3.3$ channels. Another important finding of the latter study is that Zn^{2+} induces a significant slowing of the deactivation kinetics of the T-currents mediated by $Ca_V3.3$ channels, which results in enhanced Ca^{2+} entry through $Ca_V3.3$ channels [48]. In other words, Zn^{2+} behaves as a mixed blocker/opener of $Ca_V3.3$ channels. As a pharmacological probe, the rather highly selective block of $Ca_V3.2$ channels by Zn^{2+} is of interest to further evaluate any role of this channel isotype.

6. Next steps towards more selective T-channel blockers

6.1 From mibefradil to new T-channel blockers

A well known example of a molecule developed as a so-called 'T-channel blocker' is mibefradil (see Section 3.2). Considering mibefradil, as well as several other T-channel blockers, Doddareddy *et al.* [49] generated a hypothetical 3D pharmacophore model by using common feature hypothesis generation approach (HipHop). Using this pharmacophore, these authors developed virtual screening of chemical databases and selected hits that were experimentally tested for

the block of recombinant T-channels. The best compounds were then selected for the generation of a new pharmacophore, leading to the recent description of novel T-channel blockers [50,51]. Many of these compounds are potent inhibitors of T-channels with IC_{50} values in the 0.1 µM range, but it remains critical to evaluate how selective they are for T-channels [52]. Other trials to develop mibefradil-related T-channel blockers have been reported. NNC 55-0396 one of these derivatives [53], blocks $Ca_V3.1$ channels with an IC_{50} ~ 7 µM without affecting other VDCCs in insulin secreting cells. The relative selectivity of this compound is of interest. Altogether, it is expected that virtual screening and implementation of pharmacophoric models will lead to the identification of new potent and selective T-channels blockers.

6.2 From dihydropyridines to new T-channel blockers

Most dihydropyridine (DHP) antagonists are selective inhibitors of L-type calcium channels but a number of reports have described that T-channels also show sensitivity to some commonly used DHPs [54,55]. Kumar *et al.* have recently described a novel series of DHP derivatives that present significant inhibition of T-channels [56]. There is also considerable interest in another DHP analogue, efonidipine, which was developed as an antihypertensive and antianginal drug. Although the racemic mixture of efonidipine dually blocks L- and T-channels, its enantiomeric form, *R*(-) efonidipine, shows high affinity to T-channels [57]. In guinea-pig cardiomyocytes, 1 µM *R*(-) efonidipine inhibited T-current by > 80%, although having no significant effect on L-current [57]. *R*(-) efonidipine appears as a promising selective inhibitor of T-channels. Considering these later studies [56,57], especially the recent evidence describing the efficacy *R*(-) efonidipine in blocking selectively T-channels [57], as well as benidipine [58], the identification of novel and clinically active T-selective DHPs remains of particular interest.

7. Expert opinion

Although we do not yet have selective blockers for T-channels, the postcloning era is full of promise [59]. The knockout animals have revealed many aspects of the role played by T-channels in normal physiology and in several disease states. Mutations in the gene coding $Ca_V3.2$ T-channels (*CACNA1H*) associated with childhood absence epilepsy and autism spectrum disease have been identified [60]. Undoubtedly, the knowledge about the structural organisation and the active domains of T-channels, their endogenous modulation [61], and the role(s) of partners, which have yet to be identified, will be significantly enhanced in the coming years. There is a great need for selective blockers in the T-channel toolkit and the extension of the various approaches described above should help to generate such molecules soon. Considering that the Ca_V3 proteins are useful to handle in heterologous expression systems, investigations of their pharmacological profiles are being developed both at the level of academic laboratories and in pharmaceutical companies for high-throughput screening. In several years, these new T-channel blockers may also hopefully have interest in clinical use. The authors strongly believe that inhibition of T-channels hold a major therapeutic interest in lowering heart rate, decreasing blood pressure improving coronary flow and treating severe pain and epilepsy.

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. HUGUENARD JR: Low-threshold calcium currents in central nervous system neurons. *Ann. Rev. Physiol.* (1996) 58:329-348.
2. PEREZ-REYES E: Molecular physiology of low-voltage-activated T-type calcium channels. *Physiol. Rev.* (2003) 83:117-161.
3. YUNKER AM, MCENERY MW: Low-voltage-activated ('T-Type') calcium channels in review. *J. Bioenerg. Biomembr.* (2003) 35:533-575.
4. LACINOVA L: Voltage-dependent calcium channels. *Gen. Physiol. Biophys.* (2005) 24(Suppl. 1):1-78.
5. NILIUS B, TALAVERA K, VERKHRATSKY A: T-type calcium channels: the never ending story. *Cell Calcium* (2006) 40:81-88.
•• This overview introduces a special issue of *Cell Calcium* on the molecular and physiological properties of T-channels.
6. KIM D, SONG I, KEUM S *et al.*: Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking α1G T-type Ca^{2+} channels. *Neuron* (2001) 31:35-45.
7. CHEN CC, LAMPING KG, NUNO DW *et al.*: Abnormal coronary function in mice deficient in α1H T-type Ca^{2+} channels. *Science* (2003) 302:1416-1418.
8. KIM D, PARK D, CHOI S, LEE S, SUN M, KIM C, SHIN HS: Thalamic control of visceral nociception mediated by T-type Ca^{2+} channels. *Science* (2003) 302:117-119.
9. ANDERSON MP, MOCHIZUKI T, XIE J *et al.*: Thalamic $Ca_V3.1$ T-type Ca^{2+} channel plays a crucial role in stabilizing sleep. *Proc. Natl. Acad. Sci. USA* (2005) 102:1743-1748.
10. MANGONI ME, TRABOULSIE A, LEONI AL *et al.*: Bradycardia and slowing of the atrioventricular conduction in mice lacking $Ca_V3.1$/α1G T-type calcium channels. *Circ. Res.* (2006) 98:1422-1430.

11. MISHRA SK, HERMSMEYER K: Selective inhibition of T-type Ca^{2+} channels by Ro 40-5967. *Circ. Res.* (1994) 75:144-148.

12. CLOZEL JP, ERTEL EA, ERTEL SI: Discovery and main pharmacological properties of mibefradil (Ro 40-5967), the first selective T-type calcium channel blocker. *J. Hypertens.* (1997) 15(Suppl.)S17-S25.

13. LACINOVA L: Pharmacology of recombinant low-voltage activated calcium channels. *Curr. Drug Targets CNS Neurol. Disord.* (2004) 3:105-111.

14. BEEDLE AM, HAMID J, ZAMPONI GW: Inhibition of transiently expressed low- and high-voltage-activated calcium channels by trivalent metal cations. *J. Membr. Biol.* (2002) 187:225-238.

15. LEE JH, GOMORA JC, CRIBBS LL, PEREZ-REYES E: Nickel block of three cloned T-type calcium channels: low concentrations selectively block α1H. *Biophys. J.* (1999) 77:3034-3042.

16. ERTEL SI, ERTEL EA, CLOZEL JP: T-type Ca^{2+} channels and pharmacological blockade: potential pathophysiological relevance. *Cardiovasc. Drugs Ther.* (1997) 11:723-739.

17. PO AL, ZHANG WY: What lessons can be learnt from withdrawal of mibefradil from the market? *Lancet* (1998) 351:1829-1830.

18. LEURANGUER V, MANGONI ME, NARGEOT J, RICHARD S: Inhibition of T-type and L-type calcium channels by mibefradil: physiologic and pharmacologic bases of cardiovascular effects. *J. Cardiovasc. Pharmacol.* (2001) 37:649-661.

19. MOOSMANG S, HAIDER N, BRUDERL B, WELLING A, HOFMANN F: Antihypertensive effects of the putative T-type calcium channel antagonist mibefradil are mediated by the L-type calcium channel $Ca_V1.2$. *Circ. Res.* (2006) 98:105-110.
• **A genetic dissection of the effects of mibefradil on T- and L-channels, revealing its physiological effects on L-type channels.**

20. GACKIERE F, BIDAUX G, LORY P, PREVARSKAYA N, MARIOT P: A role for voltage gated T-type calcium channels in mediating 'capacitative' calcium entry? *Cell Calcium* (2006) 39:357-366.

21. TANG CM, PRESSER F, MORAD M: Amiloride selectively blocks the low threshold (T) calcium channel. *Science* (1988) 240:213-215.

22. TYTGAT J, VEREECKE J, CARMELIET E: Mechanism of cardiac T-type Ca channel blockade by amiloride. *J. Pharmacol. Exp. Ther.* (1990) 254:546-551.

23. WILLIAMS ME, WASHBURN MS, HANS M *et al.*: Structure and functional characterization of a novel human low-voltage activated calcium channel. *J. Neurochem.* (1999) 72:791-799.

24. MONTEIL A, CHEMIN J, BOURINET E, MENNESSIER G, LORY P, NARGEOT J: Molecular and functional properties of the human α1G subunit that forms T-type calcium channels. *J. Biol. Chem.* (2000) 275:6090-6100.

25. TODOROVIC SM, LINGLE CJ: Pharmacological properties of T-type Ca^{2+} current in adult rat sensory neurons: effects of anticonvulsant and anesthetic agents. *J. Neurophysiol.* (1998) 79:240-252.

26. ARNOULT C, VILLAZ M, FLORMAN HM: Pharmacological properties of the T-type Ca^{2+} current of mouse spermatogenic cells. *Mol. Pharmacol.* (1998) 53:1104-1111.

27. BERTHIER C, MONTEIL A, LORY P, STRUBE C: α1H mRNA in single skeletal muscle fibres accounts for T-type calcium current transient expression during fetal development in mice. *J. Physiol.* (2002) 539:681-691.

28. AKAIKE N: T-type calcium channel in mammalian CNS neurones. *Comp. Biochem. Physiol. C.* (1991) 98:31-40.

29. SANTI CM, CAYABYAB FS, SUTTON KG *et al.*: Differential inhibition of T-type calcium channels by neuroleptics. *J. Neurosci.* (2002) 22:396–403.

30. RICHELSON E, SOUDER T: Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. *Life Sci.* (2000) 68:29-39.

31. HUGUENARD JR: Block of T -Type Ca^{2+} channels is an important action of succinimide antiabsence drugs. *Epilepsy Curr.* (2002) 2:49-52.

32. CRUNELLI V, LERESCHE N: Block of thalamic T-Type Ca^{2+} channels by ethosuximide is not the whole story. *Epilepsy Curr.* (2002) 2:53-56.

33. HAINSWORTH AH, MCNAUGHTON NC, PEREVERZEV A, SCHNEIDER T, RANDALL AD: Actions of sipatrigine, 202W92 and lamotrigine on R-type and T-type Ca^{2+} channel currents. *Eur. J. Pharmacol.* (2003) 467:77-80.

34. TODOROVIC SM, JEVTOVIC-TODOROVIC V, MENNERICK S, PEREZ-REYES E, ZORUMSKI CF: $Ca_V3.2$ channel is a molecular substrate for inhibition of T-type calcium currents in rat sensory neurons by nitrous oxide. *Mol. Pharmacol.* (2001) 60:603-610.

35. TODOROVIC SM, PEREZ-REYES E, LINGLE CJ: Anticonvulsants but not general anesthetics have differential blocking effects on different T-type current variants. *Mol. Pharmacol.* (2000) 58:98-108.

36. TRABOULSIE A, CHEMIN J, KUPFER E, NARGEOT J, LORY P: T-type calcium channels are inhibited by fluoxetine and its metabolite norfluoxetine. *Mol. Pharmacol.* (2006) 69:1963-1968.

37. CHUANG RS, JAFFE H, CRIBBS L, PEREZ-REYES E, SWARTZ KJ: Inhibition of T-type voltage-gated calcium channels by a new scorpion toxin. *Nat. Neurosci.* (1998) 1:668-674.

38. SIDACH SS, MINTZ IM: Kurtoxin, a gating modifier of neuronal high- and low-threshold ca channels. *J. Neurosci.* (2002) 22:2023-2034.

39. OLAMENDI-PORTUGAL T, GARCIA BI, LOPEZ-GONZALEZ I *et al.*: Two new scorpion toxins that target voltage-gated Ca^{2+} and Na^{+} channels. *Biochem. Biophys. Res. Commun.* (2002) 299:562-568.

40. DI MARZO V, DE PETROCELLIS L, BISOGNO T: The biosynthesis, fate and pharmacological properties of endocannabinoids. *Handb. Exp. Pharmacol.* (2005) 168:147-185.

41. CHEMIN J, MONTEIL A, PEREZ-REYES E, NARGEOT J, LORY P: Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. *EMBO J.* (2001) 20:7033-7040.

42. CHEMIN J, NARGEOT J, LORY P: Chemical determinants involved in anandamide-induced inhibition of T-type calcium channels. *J. Biol. Chem.* (2007) 282:2314-2323.
• **This study provides a detailed analysis of the modulation of T-channels by bioactive lipids.**

43. DANTHI SJ, ENYEART JA, ENYEART JJ: Modulation of native T-type calcium channels by omega-3 fatty acids. *Biochem. Biophys. Res. Commun.* (2005) 327:485-493.

44. TALAVERA K, STAES M, JANSSENS A, DROOGMANS G, NILIUS B : Mechanism of arachidonic acid modulation of the T-type Ca^{2+} channel α1G. *J. Gen. Physiol.* (2004) 124:225-238.
45. MATHIE A, SUTTON GL, CLARKE CE, VEALE EL: Zinc and copper: pharmacological probes and endogenous modulators of neuronal excitability *Pharmacol. Ther.* (2006) 111:567-583.
46. BUSSELBERG D, MICHAEL D, EVANS ML, CARPENTER DO, HAAS HL: Zinc (Zn^{2+}) blocks voltage gated calcium channels in cultured rat dorsal root ganglion cells. *Brain Res.* (1992) 593:77-81.
47. JEONG SW, PARK BG, PARK JY, LEE JW, LEE JH: Divalent metals differentially block cloned T-type calcium channels. *Neuroreport* (2003) 14:1537-1540.
48. TRABOULSIE A, CHEMIN J, CHEVALIER M, QUIGNARD JF, NARGEOT J, LORY P : Subunit-specific modulation of T-type calcium channels by zinc. *J. Physiol.* (2007) 578:159-171.
49. DODDAREDDY MR, JUNG HK, LEE JY *et al.*: First pharmacophoric hypothesis for T-type calcium channel blockers. *Bioorg. Med. Chem.* (2004) 12:1605-1611.
•• **This article is the first of a series of study dedicated to the search of novel T-channel blockers.**
50. CHOI JY, SEO HN, LEE MJ *et al.*: Synthesis and biological evaluation of novel T-type calcium channel blockers. *Bioorg. Med. Chem. Lett.* (2007) 17:471-475.
51. DODDAREDDY MR, CHOO H, CHO YS *et al.*: 3D pharmacophore based virtual screening of T-type calcium channel blockers. *Bioorg Med Chem* (2007) 15:1091-1105.
52. PARK JH, CHOI JK, LEE E *et al.*: Lead discovery and optimization of T-type calcium channel blockers. *Bioorg. Med. Chem.* (2007) 15:1409-1419.
53. HUANG L, KEYSER BM, TAGMOSE TM *et al.*: NNC 55-0396 [(1S,2S)-2-(2-(*N*-[(3-benzimidazol-2-yl) propyl]-*N*-methylamino)ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl cyclopropanecarboxylate dihydrochloride]: a new selective inhibitor of T-type calcium channels. *J. Pharmacol. Exp. Ther.* (2004) 309:193-199.
54. AKAIKE N, KOSTYUK PG, OSIPCHUK YV: Dihydropyridine-sensitive low-threshold calcium channels in isolated rat hypothalamic neurones. *J. Physiol.* (1989) 412:181-195.
55. GODFRAIND T: Calcium-channel modulators for cardiovascular disease. *Expert Opin. Emerg. Drugs* (2006) 11:49-73.
56. KUMAR PP, STOTZ SC, PARAMASHIVAPPA R, BEEDLE AM, ZAMPONI GW, RAO AS: Synthesis and evaluation of a new class of nifedipine analogs with T-type calcium channel blocking activity. *Mol. Pharmacol.* (2002) 61:649-658.
57. TANAKA H, KOMIKADO C, SHIMADA H *et al.*: The *R*(-)-enantiomer of efonidipine blocks T-type but not L-type calcium current in guinea pig ventricular myocardium. *J. Pharmacol. Sci.* (2004) 96:499-501.
• **Describes *R*(-) efonidipine as a promising selective T-channel blocker (see also [58]).**
58. FURUKAWA T, NUKADA T, MIURA R *et al.*: Differential blocking action of dihydropyridine Ca^{2+} antagonists on a T-type Ca^{2+} channel (α1G) expressed in *Xenopus* oocytes. *J. Cardiovasc. Pharmacol.* (2005) 45:241-246.
59. MCGIVERN JG: Pharmacology and drug discovery for T-type calcium channels. *CNS Neurol. Disord. Drug Targets* (2006) 5:587-603.
60. BIDAUD I, MEZGHRANI A, SWAYNE LA, MONTEIL A, LORY P: Voltage-gated calcium channels in genetic diseases. *Biochim. Biophys. Acta* (2006) 1763:1169-1174.
61. CHEMIN J, TRABOULSIE A, LORY P: Molecular pathways underlying the modulation of T-type calcium channels by neurotransmitters and hormones. *Cell Calcium* (2006) 40:121-134.

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